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Cytotoxic Effect of Aqueous Stem Bark Extract of *Vitellaria Paradoxa* on Human Peripheral Blood Mononuclear Cells

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Abstract
Background*: Vitellaria paradoxa is a popular tree with several applications in folkloric medicine. Concoction of different parts of the V. paradoxa have been used in the treatment of bacterial, viral and fungal infections. Peripheral blood mononuclear cells (PBMCs) are the central cells of the immune system and are responsible for adaptive immunity. This study evaluated the cytotoxic effect of aqueous stem bark extract of V. paradoxa on PBMCs.*
Materials and Methods: *Thirty (30) apparently healthy volunteers were recruited for this study. PBMCs were harvested using Histopaque 1077 and suspended in 2ml Rose well Park Memorial Institute culture Media (RPMI-1640). The cells were treated with various concentrations (25µg/ml, 50µg/ml, 100µg/ml and 1000µg/ml) of V. paradoxa stem bark extract. Cells Viability was determined using trypan blue exclusion method.*
Results and Conclusion: *The result indicated that the level of Cytotoxicity increase concurrently with the increase in concentration of the extract and shows statistical significant ($P < 0.05$) differences in each case, hence, phytochemical analysis in order to identify the cytotoxic component is highly recommended and avoidance of excess consumption of V. paradoxa concoction is highly encouraged*
Key words: *Cytotoxicity, PBMCs, Viability, Vitellaria paradoxa*

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I. Introduction

Peripheral blood mononuclear cells (PBMCs) are the central cells of the immune system; they are responsible for adaptive and innate immunity.[1] In human body; Lymphocytes, Monocyte, and Dendritic cells continuously circulate in blood and lymph and are capable of migrating into tissue spaces and lymphoid organs in order to destroy the invading pathogen.[2] PBMCs are single round nuclei cells comprising of T cells (70%), B cells (15%), Monocytes (5%), Dendritic cells (1%) and Natural killer (NK) cells (10%).[3,4] Medicinal plants are used globally to cure, treat and prevent sicknesses and injuries to people, out of 422, 000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes.[5] *V. paradoxa* (Shea butter) is a popular tree with several applications in folkloric medicine the tree grows naturally in the wild of dry savannah belt of West Africa and stretches in abundance up to the foothills of Ethiopian mountain.[6] The stem bark, roots, fruits, kernel, leaves and flower of *V. paradoxa* was reported to have a great medicinal value, particularly in the preparation of skin ointment, It promotes wound healing and soothes skin irritation, also used to treat inflammation, rashes in children, dermatitis, chapping and ulcers, as well as rub for rheumatism.[6] Bark decoction of *V. paradoxa* is used to facilitate child birth and to encourage lactation after delivery, or as a footbath neutralizes venom of the spitting cobra.[7] Similarly, Leaf, Root and Bark of *V. paradoxa* have been used to cure many infectious diseases in different parts of Nigeria.[8,9] The *V. paradoxa* tree produces latex. This latex is used traditionally in a mixture with palm oil to produce glue. The shell or husks of the shea nut is used in the purification of water. It has the ability to remove substantial amounts of heavy metal from aqueous solutions. [8] The shell is pounded and made into paste that is used in northern Ghana for plastering traditional mud houses. Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections.[10] According to El-Mahmood[11] Preliminary phytochemical screening of the stem

bark extracts of *V. paradoxa* revealed the presence of carbohydrates, alkaloids, saponins, tannins and cardiac glycosides. Ethanol, acetone and aqueous extracts of the plant inhibit the growth of pathogenic *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Shigella dysenterie* and *Salmonella typhi* with varying degrees of activity with the ethanol extracts demonstrating the highest activity against all the test organisms[11]. People consume extract of *V. paradoxa* for treatment of many bacterial, Viral and fungal infections. But actually, they don't know the exact concentration they are taking. The extract may cause toxic effect to their biological system, especially the cells and organs of immune system.[12] As such it is important to check the toxicity and safety of *V. paradoxa* on human peripheral blood mononuclear cells (PBMC).

II. Materials And Methods

Study Area

The study was carried out in the Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria

Study Subjects

A total of thirty (30) apparently healthy volunteers comprising of 15 males and 15 females were recruited for this study. Their age ranges from 20-30 years. The participants signed an informed consent and had no active disease at the time of phlebotomy.

Plant collection and identification

The plant was collected locally. The stem bark, fruits and flower were identified at the Herbarium section of Botany Unit, Department of Biological Sciences, Faculty of Sciences, UDUS, and the voucher identification number issued was UDUS/ANS/0248.

Preparation of Plant material

The stem bark was cut into small pieces and air-dried under shade at room temperature for 10 days. Subsequently, it was grinded into powder with a pestle and mortar. About 600 g of the powdered stem bark was cold macerated with 2500 ml of freshly prepared distilled water exhaustively for 24 hours with occasional shaking. The resultant mixture was filtered. The filtrate was evaporated to dryness in a hot air oven at a temperature of 50^{o}C giving brown residue called "crude extract" of *V. paradoxa.* The crude extract was stored in a refrigerator until required for use.[13]

Collection of Blood and Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Three milliliter (3ml) of venous blood was aseptically collected from each participant and dispensed in Heparinized tube. The sample was mix thoroughly and carefully layered on Histopaque-1077 (Sigma Aldrich, UK) without getting mixed up, it was spun down at 400g for 30 minutes. After centrifugation, the upper layer was aspirated and discarded. The opaque interface containing PBMC was carefully transferred with a Pasteur pipette into a clean conical centrifuge tube. The cells were mixed with 10 ml isotonic phosphate buffer the washing was repeated 3 times at 250g for 10 minutes, the supernatant was discarded. The cells were suspended in 2 ml RPMI-1640 (Sigma Aldrich UK).

Cell Count (Pre-treatment cells count)

In order to ascertain the number of viable cells, the PBMCs suspension was counted as follows, 10µl of 0.4% Trypan Blue solution (w/v) and equal volume of the PBMCs suspension were placed into 2ml Eppendorf tube and mixed thoroughly, it was allowed to stand for 5min at room temperature. The neubeaur counting chamber was charged with the mixture. Viable and Non-viable PBMCs were counted accordingly. Only PBMCs suspension that contains 90% and above, viable cells was exposed to different concentration of extract.

Treatment of PBMCs with Plant Extract

Five (5) falcon tubes were placed in a tube rack, into each tube 150µl of PBMCs suspension (1 x 10^{6}cells/ml) was dispensed, followed by addition of various concentration(25µg/ml, 50µg/ml, 100µg/ml and 1000 µg/ml) of plant extract into the tubes respectively. The tubes were labeled appropriately and incubated at 37°C for 30 minutes. PBMCs suspension without plant extract was considered as control. All the treatments were performed in duplicate and the mean was calculated in each case

Cells viability test (Trypan blue exclusion method)

Ten microliter (10µl) of treated PBMCs suspension and 10µl of 0.4% trypan blue were mixed in 2ml Eppendorf tube, and left for 10minutes at room temperature. Cleaned, dried haemocytomter was charged with the mixture and observed under microscope. Both viable and non-viable cells were counted. Normal cell viability was considered to be 90–95% colorless cells. The percentage of both viable and non-viable cells were counted.[13]

Calculations

Percent Viability = $\frac{\text{Total number of viable cells}}{\text{Total number of cells (Viable and Non-viable)}} \times 100$

III. Data Analysis

The data was analyzed using SPSS 21 Software package (IBM, USA). Test for normality was performed to ascertain normal distribution of the variables. Data was normally distributed based on tests of normality results: as such parametric test was carried out. More than two groups were analyzed using analysis of variance (ANOVA). Where by Post-Hoc; Benferroni multiple comparison test was carried out. The results were expressed as mean ± standard error of mean. The p value ≤ 0.05 was used to determine the level of statistical significance

IV. Results

Total PBMC Count and Percentage Viability

The mean (SEM) of total viable cell count (TVCC) before treatment was 288.2 x 10^6 cells/ml (SEM = 5.39). Among the treatment, the PBMCs treated with 1000 µg /ml of the stem bark extract shows the lowest mean score of viable cells count of 86.90 x 10^6 cells/ml (SEM = 5.35). The total non-viable cells count (TNVCC) result shows that human PBMCs treated with 1000 µg/ml of the stem bark extract have the highest mean score of non-viable cells 48.20 x 10^6 cells/ml (SEM = 4.3). Mean percentage viability of PBMCs before treatment was 94.25%. PBMCs treated with highest concentration (1000 µg /ml) of the extract recorded a lowest mean percentage score of viability 65.47% (Table 1).

The result presented in Table 2 shows the effect of different concentrations of stem bark extract of *V. paradoxa* on viability of Peripheral Blood Mononuclear Cells (PBMC). The mean percentage viability between the groups, after treatment with different concentration of *V. paradoxa* stem bark extract (25µg/ml, 50 µg/ml, 100µg/ml and 1000µg/ml), a statistical significant (P>0.05) differences was observed in some cases.

The result in table 3 shows comparisons of mean Parentage viable Pretreatment group with mean Parentage viable of control group and mean Parentage viable of treated groups, the result shows non-statistically significant (P > 0.05) difference on comparison of PVP group with control. When PVP group was compared with treated groups a statistically (P <0.0001) significant difference was observed.

Table 1: Total Peripheral Blood Mononuclear Cells count and percentage viability before and after treatment with stem back extract of *V. paradoxa*

Conc. (µg/ml)	TVCC x10^6 cells/ ml		TNVCC x10^6 cells/ ml		Percentage Viability	
	Mean	SEM	Mean	SEM	Mean %	SEM
Pre-treatment	288.2	5.39	17.50	1.71	94.25	0.54
Control	263.7	10.27	16.70	1.86	93.80	0.64
25 µg/ml	152.8	5.68	17.02	2.39	90.83	1.08
50 µg/ml	147.9	4.98	17.00	1.76	90.06	1.02
100 µg/ml	148.4	7.55	17.43	1.82	87.95	1.29
1000 µg/ml	86.9	5.35	48.20	4.3	65.47	2.06

TVCC = Total viable cell count, TNVCC = Total non-viable cell count

Table 2: Effect of different concentrations of stem bark extract of *V. paradoxa* on viability of Peripheral Blood Mononuclear Cells (PBMC)

Conc. (µg/ml)	Mean viability (%)	ANOVA (Bonferonni)				
		F-value	p-value	Compared mean	MD	p-value
Control	93.86	59.18	0.000	Control vs. 25 µg	2.9	>0.05
25	90.83			Control vs. 50 µg	3.7	0.77
50	90.06			Control vs. 100 µg	5.8	0.06
100	87.95			Control vs. 1000 µg	28.3	<0.0001
1000	65.47					

MD = Mean Differences

Table 3: comparisons of mean Percentage Viable Pretreatment group with Percentage Viable Treated groups

Paired variables	Percentage Mean viability (%)	Mean Difference	S EM	p-value
PVP vs. PV Control	94.25 vs. 93.80	0.44	3.10	0.44
PVP vs. PV 25 µg/ml	94.25 vs. 90.83	3.41	5.10	0.00
PVP vs. PV 50 µg/ml	94.25 vs. 90.06	4.18	5.49	0.00
PVP vs. PV 100 µg/ml	94.25 vs. 87.95	6.29	6.90	0.00
PVP vs. PV 1000 µg/ml	94.25 vs. 65.47	8.77	13.95	0.00

PVP = percentage viable pretreatment, PV = percentage viable, SEM = **Standard Error of Mean**

V. Discussion

When normal cells are treated with any toxic compound, they may undergo necrosis due to lose of membrane integrity and rapid death occurs as a result of cell lysis.[14] In this study, thirty (30) apparently healthy volunteers participated. Among which are 15 males and 15 females with aged 20 and 30 years. In this study we found that the mean percentage viability of pretreated PBMC was 94.25% and this was considered to be within the reported range as the normal cell viability was 90–95% colorless cells.[15] This study shows that, the viability of PBMC treated with different concentrations of the stem bark extract was found to be decreasing as the concentration of the extract increases. This suggests high concentration of the extract has more effect to the PBMC survival. Exposure of normal cells to these compounds may lead to apoptosis or incidental cell death.[16] previous study reported that prolong administration of stem bark extract of *V. paradoxa* causes a decrease in white blood cells count.[17] Phytochemical screening of the root, stem bark and leaves of *V. paradoxa* revealed the presence of Carbohydrates, simple reducing sugars, soluble starch, Saponins, Alkaloids and Tannins. [16,8,9] This study further justify the effect of *V. paradoxa* on PBMC by pairing pre-treated PBMCs with those exposed to different concentration of the stem bark extract of the plant and control was included. Our findings shows that pre-treated PBMC and control did not differ however the differences arise when pre-treated PBMCs were paired with treated ones. This confirms the effect and rule out confounding factors that may influence the result. This study demonstrated that, the aqueous stem back extract of *V paradoxa* have significant cytotoxic effects on PBMCs at high concentration (1000 µg/ml), which is confirmed by trypan blue dye exclusion assay. This is in line with the earlier report by Ayankunle *et al*, who reported that toxic effect of both the aqueous and methanolic stem bark extract of *V paradoxa* has significantly altered the platelet count in rats treated with high concentration (50mg/ml) of the extract.[17] Similarly, our result findings is in line with Sudeep *et al*, who reported cytotoxic effects of three different extract on cultured lymphocytes from human.[13] However, the result of this study is contrary to previous report by Mainasara *et al,* who reported the absence of death or signs of toxicity in wistars rats after 1 to 14 days post exposure to different concentration of *V. paradoxa* stem bark extract.[19] This nonconformity might be attributed to the fact that, different parameters [Albumin (ALB), total protein (TP), total and conjugated Bilirubin (TB and CB), amino transferases (AST and ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT)], were used to assess the hepatotoxicity of the extract. It might also be as a result of the ability of hepatocytes to resist toxicity compared to the other immune cells of the body, due to the fact that the liver is the central organ for detoxification.[20]

VI. Conclusion

It's clear that high concentration of aqueous stem back extract of *Vitellaria paradoxa* has cytotoxic effect to human peripheral blood mononuclear cells (PBMC). There for, Public awareness and enlightenment to avoid taking excess concoction of *Vitellaria paradoxa* for treatment of different ailment such bacterial, fungal, parasitic infections among others is highly recommended.

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References

[1]. Murphy, K. and Weaver, C. The Humoral Immune Response". *Immunobiology*; 2016 **1**(9): Garland Science. ISBN 978-0-8153-4505-3
[2]. Hodes, R. J. *Immunology Review*; 1997; **160**: 5-8.
[3]. Autissier, P., Soulas, C., Burdo, T. H. and Williams, K. C. Evaluation of a 12-color flow cytometry panel to study Lymphocyte, Monocyte, and Dendritic cell subsets in humans. *Cytometry*; 2010; **77**: 410–419. doi: 10.1002/cyto.a.20859
[4]. Ahmed, R. N., Sani A. and Igunnugbemi, O. O. Antifungal Profile of *Vitellaria paradaxa* stem bark extract. *Journal of Chemical and Pharmaceutical Research* Review; 2009; **9**(2): 25-33.
[5]. Schippmann, U., Leaman, D. J. and Cunningham, A. B. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity; Global Trends and Issues. In (FAO). *Biodiveristy and the ecosystem approach in agriculture, forestry and fisheries;* 2002; **3**: 26-45.
[6]. Adamu, H. M., Usher O. A. and Nansen, E. Antimicrobial activity of oil from *butyric premium parkii* seed (Shea butter). *International Journal of Modern Biology and Medicine;* 2013; **3**(2): 50-59.
[7]. Hall, J. B., Aebisher, D. P., Tomlinson, H. F., Osei- Amaning, E. and Hindle, H. R. *Vitellaria paradoxa*: A monograph. School of Agriculture and Forest Sciences; publication, University of Wales; 1996; 5-6
[8]. Falana MB, Bankole, MO, Ojo DA, Omemu AM. Comparative Phytochemical Investigation of the Various Parts of *Vitellaria Paradoxa. Journal of Natural Science Research*. 2016; **6**(11): 74-80.
[9]. Falana, M. B., Bankole, M. O., Ojo, D. A., Omemu, A. M. and Bankole,S. O. Efficacy and Safety of Leaf, Root and Stem bark extract of *Vitellaria Paradoxa* on Diarrhea induced albino Rats, *American Journal of Microbiological Research;* 2016; **4**(3): 73-76.
[10]. Soladoye, M. O, Orhiere S. S.andIbimode, B. M. Ethanobotanical Study of two Indigenous Multipurpose Plants in the Guinea Savanna of Kwara State - *Vitellaria paradoxa* and *Parkiabiglobosa. Biennial Conference of Ecological Society of Nigeria*; 1989; **4**: 21-32.

[11]. El-Mahmood, A. M., Doughari, J. H. and Ladan, N. Antimicrobial screening of stem bark extract of *Vitelleria paradoxa* against some enteric pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*; 2008; **2**(5): 89-94.
[12]. Otuki, M. F., Vieira-Lima, F.,Malheiros, A., Yunes, R. A. and Calixto, J. B. Topical anti-inflammatory effects of the ether extract from Protiumkleinii and α-amyrinpentacyclictriterpene. *European Journal of Pharmacology*; 2005; **507**(1): 253-259.
[13]. Sudeep, N., Niyhya, M. N and Kiranmayee, P. Evaluation of cytotoxic effects of plants extract on PBMCs. *Journal of Chemistry and Pharmaceutical Research*; 2017; **9**(7): 18-26.
[14]. Hangartner, M. O. Program cell death. *Cold spring Harbor Laboratory press*; 1997; **5**: 384-496.
[15]. Chahra B, Bouriche H, Eduardo OE, Senator A. Immunomodulatory effects of *Santolina chamaecyparissus* leaf extracts on human Neutrophil Functions, Pharmaceutical Biology, 2016; **54** (4); 667-673.
[16]. Niles AL, Moravec RA, Eric Hesselberth P, Scurria MA, Daily WJ, Riss TL. A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. *Analytical Biochemistry*.2007; **366**(2):197-206.
[17]. AyankulneA. A., Kolawola, O. T., Adesokan, A. A. and Akiiibinu M. O. Antibacterial activity and subchronic toxicity of *Vitellaria paradoxa* stem bark extract. *Journal of Pharmacology and Toxicology*; 2012; 7: 298-304
[18]. Ndukwe IG, Amupitan JO, Isah Y, Adegoke KS. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa*. *African Journal of Biotechnology* 2007; **6** (16): 1905-1909.
[19]. Mainasara, A. S., Oduola, T., Musa, U., Mshelia, A. S. and Muhammad, A. O. Hepatotoxicity assessment of *Vitellaria paradoxa* stem bark extract in Wister rats. *European journal of medicinal plants*; 2016; **13**(3): 1-9.
[20]. Giannin A. Contribution of "Women's Gold" to West African Livelihoods: The Case of Shea (*Vitellaria paradoxa*) in Burkina Faso. *Economic Botany*; 2005; **66**: 237-248.

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